

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
27 September 2001 (27.09.2001)

PCT

(10) International Publication Number
WO 01/70258 A1

(51) International Patent Classification⁷: **A61K 38/47,**
38/48, 31/355

Guimarães, 176, Pacaembú, CEP-01250-000 São Paulo, SP (BR). **DE NUCCI, Gilberto** [BR/BR]; Av. Jesuino Marcondes Machado 415, Campinas, CEP-13092-320 São Paulo, SP (BR). **FALCI, Marcio** [BR/BR]; Av. Marechal Hastinphilo de Moura, 338, G-12A, CEP-05641-900 São Paulo, SP (BR).

(21) International Application Number: **PCT/BR01/00026**

(22) International Filing Date: 19 March 2001 (19.03.2001)

(25) Filing Language: English

(74) Agent: **CRUZEIRO/NEWMARC PATENTES E MARCAS LTDA.**; Rua Itajobi, 79, Pacaembu, CEP-01246-010 São Paulo, SP (BR).

(26) Publication Language: English

(71) Applicant (for all designated States except US): **TOPIC EMPREENDIMENTOS E PARTICIPAÇÕES S/C LTDA.** [BR/BR]; Calçada das Bétulas, 48 - Conj. 4, Centro Comercial Alphaville, CEP-06453-000 Barueri, SP (BR).

(81) Designated States (national): AU, CA, CN, CZ, HU, ID, IL, IN, IS, JP, KR, MX, NO, NZ, PL, TR, US.

Published:

— with international search report

(72) Inventors; and

(75) Inventors/Applicants (for US only): **SANTANA, Cristiano, Alberto, Ribeiro** [BR/BR]; Rua Almirante P.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/70258 A1

(54) Title: **PHARMACEUTICAL COMPOSITION OF CARRIER SUBSTANCE FOR PRODUCTS BASED ON VITAMIN-E, BROMELINE AND HYALURONIDASE**

(57) Abstract: The present patent application of an invention refers to a new PHARMACEUTICAL COMPOSITION OF CARRIER SUBSTANCE FOR PRODUCTS BASED ON VITAMIN-E, BROMELINE AND HYALURONIDASE, comprising the formulation: BROMELINE more than 0.1 %; HYALURONIDASE 50 to 900 utr/mg; VITAMIN-E 10 to 2000 mg.

**"PHARMACEUTICAL COMPOSITION OF CARRIER
SUBSTANCE FOR PRODUCTS BASED ON VITAMIN-E, BROMELINE AND
HYALURONIDASE"**

The present invention refers to a new
5 pharmaceutical composition applicable under any form, most
notably gel, cream, liquid, spray, aerosol, lyophilized of a
carrier substance for products to aggregate vitamin-E,
bromeline and hyaluronidase. The above mentioned
pharmaceutical composition is of topical application, non-
10 toxic and features a high penetration rate through the skin.

BRIEF DESCRIPTION OF THE INVENTION

The skin permeability varies according to the
region of the body, being the skin folds and the face those
that present the highest absorption rate. A product applied
15 over the skin will present a longer period of contact and
percutaneous absorption.

According to the classic book "Histologia dos
epitélios", by Walter A. Hadler and Sineli R. Silveira,
Editora Campus, Campinas, 1993, it is considered that:
20 "bearing in mind the general morphological characteristics
and the specialized functions that they perform, the
epithelium cells are predominantly classified into two
categories, which correspond to two epithelium classes:
coating epithelium cells and secreting epithelium cells. The
25 cells of these two classes mix with each other to constitute,
respectively, the coating epithelium and the secreting

epithelium, each one of them performing specific functions that are inherent to them. Such division is also fundamented in the distribution of these two classes of epithelium in the organism, which although wide is distinctive for both. With the purpose of forming the coating epitheliums the epithelium cells associate side-by-side, so as to originate "membranes" or layers superimposed over the base membrane, which function is to coat surfaces. On the contrary, the secreting cells unite to form organized functional units, better suited for performing their specialized function, related to the secretion products synthesis; thus are constituted the secreting units. The coating epitheliums are defined as living membranes, usually featuring a discontinuity, that isolate the organism from the environment, separating the internal media from the external one. Furthermore, these epitheliums isolate from each other the various internal media compartments, among which are the intravascular compartment, the serum compartment and several others. Among the various functions performed by the coating epitheliums some are performed by specialized variants that are specifically adapted to perform one or more functions. Others are incorporated as general functions presented without distinction by every coating epithelium cell. The coating epithelium cell, in the same way as most of the living cells, passively absorbs water and electrolytes and eliminates them actively; this function is well developed in the epithelium

cells. On that account it is very important to observe that generally it is understood as absorption the penetration of solutions through the cells plasmatic membrane. However two different specific forms of absorption must be distinguished

5 from one another: the passive absorption, that occurs according to the osmotic laws, and the active absorption, that entails the effective participation of the epithelium cell and that does not follow such physic laws. On the other hand it must be considered that every single substance that

10 penetrates the interior of a multi-cellular organism, or else is excreted or eliminated, must cross at least one coating epithelium, because every superior organism is penetrated internally and externally by epitheliums. It must also be observed that the coating epitheliums, although continuously

15 covering and protecting those surfaces it coats, are not impervious at all; that is why they do not behave as inert "membranes". On the contrary, they allow for the exchange of gases, water, several kinds of electrolytes and certain other solutes between the internal and the external media, or

20 between the various internal compartments, which characterizes its permeability. The coating epithelium cells limit in a controlled and selective way the permeability of the respective epitheliums, with the purpose of protecting the organism and still participate of the control of its

25 homeostasis. In order to perform such function the epitheliums are organized and arrange their cells in a

special form, in order to build up coatings which cells abut the base membrane and are united with each other by means of intracellular junctions; in turn the cells are coated by the plasmatic membrane, which features special characteristics, and by the glycochalice, both able to express well defined functional properties. The functional characteristics expressed by the plasmatic membrane portion that coats the cells apical surface are different from those expressed by the portion situated in its basal or basolateral face; such differences, which occur mainly on the functional aspect, contribute for the remarkable degree of polarization expressed by the coating epithelium cells. The prime function performed by the coating epitheliums correspond essentially to the protection rendered to the surface that they coat, characterizing their protective coating function. Such function features a special characteristic, being a coating that, besides offering mechanical, physical and chemical protection to the coated surface, is not inert. The coating epitheliums are pervious, which allows for the controlled and selective passage of several products through its wall. There are many evidences in favor of the idea that the coating epitheliums permeability constitutes a fundamental property, with significant functional expression, for it is essential for the performance of several functions featured by the epitheliums, even more so because it is selective and its permeability degree presents a wide variation. It is fairly

well demonstrated that the permeability degree influences strongly the function performed by the coating epitheliums:

- 1) wide permeability;
- 2) reduced permeability and
- 3) absence of permeability.

When there is a wide permeability, the epitheliums allow intense metabolic exchanges through their walls, with poor control and selectivity of its permeability. In these circumstances the epithelium acts on the filtration and transfer of metabolytes, these functions requiring little qualitative control; the exercise of these functions is subordinated to the epithelium intrinsic structure, which is adapted to act, mainly passively, being low the level of selective permeability. The coating epitheliums with a reduced degree of permeability, due to the characteristic that is so peculiar to them, present the property of partially controlling their own permeability, and above all their selectivity. As a consequence, these coating epitheliums present selective permeability, which allows them to interfere and qualitatively control their functional activity, as well as making them more able to actuate over the homeostasis control. The absence of epithelium permeability is correlated to the complex isolation of the coated surface and, on the other hand, to the better controlling of this epithelium function, because its cells, although very poorly pervious, present selective

permeability. In this case the coated surface has its boundaries limited by a "membrane" impervious or very poorly pervious and very effective, that performs an important protective function, for it is able to discriminate exactly what can cross the epithelium. The coating epitheliums permeability is such an expressive functional property that it has been used as an important classification criterion to rank them in three classes:

- 1) pervious epitheliums;
- 2) poorly pervious epitheliums and
- 3) impervious epitheliums.

Because of their selective permeability, even in the inferior animals the epitheliums have assumed the function of coating the organism, constituting its external coating, with limiting and protective properties, not only morphological but also functional. Their cells, in principle very similar, behaved as a semi-pervious "membrane" poorly effective that acted passively, but which function allowed the separation, tough precarious and more morphological than functional, between the internal and the external media. It seem to be that the majority of the coating epitheliums acts as a barrier that prevents the free passive diffusion, because their permeability, which is selective, is conditioned to several factors among which stands out the electric potential present in their cells plasmatic membrane. The continuity of the epithelium coating is established as

much through the intimate abutment of adjacent cells as through the presence of intercellular union devices. The epithelium cells are enveloped by the glycochalice, that also takes part of the coating function performed by the
5. epithelium, in addition to aid the union between adjacent cells, because the intracellular adhesive is formed also by the glycochalice. Several experimental investigations confirm that the coating epitheliums selective permeability is associated to other specific functions expressed by their
10 cells, namely: absorption, excretion and secretion. These functions, beyond their permeability, which constitutes their prime function, are responsible by the general functioning of the epithelium cell. The general functions performed by the coating epitheliums are basically the following:

- 15 1) surfaces protective coating function;
- 2) isolation and functional individualization of the internal media and of its distinct compartments, due to their cells selective permeability;
- 3) controlling the homeostasis of the
20 internal medium and its compartments due to their cells ability to interfere in the epithelium selective permeability; the epithelium cells manifest the capacity to effect the absorption, secretion and excretion; such functions interfere on the epithelium permeability;
- 25 4) performance of the metabolic functions due to their ability to effect hydrosalinic exchanges and to

effect metabolytes transfers due to their cells and intracellular spaces high degree of poorly selective permeability;

5) transport of products along the epithelial surface due to the participation of the cilia;

6) sensorial perception and

7) germinative function.

Among these functions, the first four derive mostly from the epithelium cells selective permeability, over which are additionally superimposed the additional affects corresponding to their properties of absorption, excretion and secretion. Among the general functions performed by the coating epitheliums, the selective permeability is responsible by the efficiency regarding the ability to coat, protect and isolate the surfaces, as well as to effect the control of the homeostasis; the passive absorption and the metabolytes transfer capacity are executed normally by the majority of the cells of these epitheliums, which demand only minor adaptations to become able to effectively perform such functions. On the contrary, the functions of absorption, excretion and secretion depend of properties that develop successively and would become paramount, mostly in some specialized types of coating epithelium, which adapted following a new and specific direction. The sensorial perception and the germinative function are more specific functions that are only manifest by certain epitheliums even

more specialized. Considering their cell's morphological characteristics, the coating epitheliums have been classified according to the same number of cellular extracts they bear in: simple (a single extract) and stratified (two or more
5 extracts). Both the simple epitheliums and the stratified ones, conforming to their cells format, are in turn subdivided into pavementous, cubic or prismatic. The simple epitheliums are usually adapted to manifest wholly their most expressive fundamental property that consists in their
10 permeability, which degree and selectivity vary. The simple coating epitheliums, constituted by a single layer of pavementous or cubic-prismatic cells, present major differences regarding their functional properties, correlated not only to their cell's morphology, but also to the
15 intracellular space's properties. The simple pavementous epitheliums are usually very pervious; the cubic-prismatic ones are less pervious. The coating epitheliums permeability, in addition to being selective, is controlled by their cell's functional activity, although the control loses efficiency
20 in the same order as the intracellular space's permeability increases. The cubic-prismatic epitheliums, being less pervious than the pavementous, are more effective to control their permeability. Based on the format of the epithelium cell, in its permeability and the coating epitheliums most
25 common adaptations, it is possible to generate a provisional classification for these epitheliums. Thus, the simple

coating epitheliums are divided into two classes: pavimentous and cubic-prismatic. Each class is subdivided according to its functional properties in open or pervious epitheliums, in semi-occlusive or poorly-pervious and occlusive or
5 impervious. In the simple coating epitheliums classification, the cubic epitheliums and the prismatic epitheliums are usually considered distinct, being defined and identified according to the format of the epithelium cells that make them up. However some functional studies have showed that the
10 correlation between form and function presents several exceptions. For this reason a functional classification is adopted considering predominantly it's permeability. According to this criterion these epitheliums are denominated cubic-prismatic comprising the semi-occlusive and occlusive
15 epitheliums. Following the same criterion the stratified epitheliums can be subdivided into: pavementous and cubic-prismatic. The stratified epitheliums are adapted to perform primarily the mechanical protection function, because they are impervious or poorly pervious. The epitheliums comprise,
20 in addition to the cells, the intercellular space and the base membrane, which interfere in their permeability degree; their permeability derives not only from their cell's peculiar properties, responsible for the transcellular permeability way, but also from the presence of another
25 permeability way of their walls, constituting the intercellular or paracellular way. The transcellular way

comprises two different ways that consist of the transmembranous way and the transcannular or transcitose way. It has been demonstrated, experimentally, that the coating epitheliums can be transposed by water and by substances of various natures, both through their epithelium cells (transcellular way) and through the way situated between their cells (intercellular way). In the first instance the epithelium cell can effect the permeability control of the epithelium through its biological activity, making this process selective. As for the intercellular way permeability, the epithelium cell, although not behaving in a totally passive form, does not interfere directly in the transport selectivity. The sole form of cell active participation, in this instance, comprises the determination, exceptionally, the enlargement of the corresponding intercellular space. By means of the action of the microfilaments that constitute its cito-skeleton, the epithelium cell, specially those of certain types of simple coating epitheliums pavementous of the open type, can change its format and retract segments of its cytoplasm; thus being able to influence the size of the intercellular space and regulate it. It has been established that the transcellular permeability of the simple coating epitheliums is perfectly distinct from the intercellular permeability, because both are subordinated to very different mechanisms. The epithelium cell permeability, which is selective, is influenced by its biological activity; on the

contrary, the intercellular permeability is totally passive, and thus is not selective. Several experimental results have confirmed that the transposition of solutions through the epitheliums is subject to multiple control mechanisms, among
5 which is paramount the intrinsic functional activity of its cells. On the contrary, the intercellular space permeability is generally not controlled, because in this case the transposition of a molecule through the epithelium follows only the corresponding physical laws and is directly related
10 to its diameter, its electrical cargo and, obviously, to the intercellular space size; these three variables constitute the main limiting factors that interfere on the intercellular permeability of the simple coating epitheliums. The transcellular permeability of the simple coating epitheliums
15 can be exercised through two distinct and independent ways: the transmembrane way, which is the true transcellular way, and the transcannular way, which happens through the vesicles and the cannules or tubes of the vesicle-cannular system, found inside the cytoplasm of many types of coating
20 epithelium cells". Consequently, the coating epitheliums are pervious, which allows the controlled and selective passage of various products through its wall. It is demonstrated that the permeability degree affects strongly the coating epitheliums function.

25 Three types of coating epitheliums are thus considered:

- 1- Of wide permeability;
- 2- Of reduced permeability;
- 3- Of null permeability.

The purpose is to prove through the
5 formulation that there is an intense metabolic exchange
demonstrating that the epithelium actuates on the transfer of
metabolytes. This penetration of substances is complete and
gradual and trespasses these epithelium layers until it
penetrates the small blood vessels, reaching the circulatory
10 current.

There is a description of the molecules to
estimate the coating epitheliums permeability. Ex.:
Hemoglobin, Ferritin, Lipoproteins and enzymes.

It is also known the transcitose on the
15 transposition of the epitheliums by the macro and micro
molecules until the vascular eye depending of their
association.

Bromeline is the generic name given to the
protheolitic enzymes found on pineapple (*Ananas comosus*
20 *Mer.*), as well as in other species of plants from the family
Bromeliaceae. The bromelines hydrolyze proteins, peptides,
esters and amides. Its specificity and use are relatively
similar to that of other proteases, such as papaine and
fycine, extracted from latex of papaya and fig, respectively.

25 Bromeline is also similar to papaine when it
comes to its activation and deactivation mechanism as a

sulphhidrilyc protease, that is, its enzymatic action depends on the sulphhidrilyc group of a cisteyne residue.

The presence of an enzyme with strong protheolytic activity in the fresh pineapple juice has been
5 known for a long time. Although the activity decreases significantly in the ripening, the levels are reasonably high in the juice extracted from the partially ripened fruit usually used in the processing.

Pineapple is the only fruit that features
10 relatively high concentrations of proteases in the ripe state. In papaya, the papaine is only found in high levels when the fruit is still green.

As raw material for the obtention of bromeline different parts of the plant can be used: leaves,
15 stems, fruit juice, skin and industrial residues. However, the most commonly used raw material is the ripe pineapple plant stems, used after the harvesting of the fruit. This raw material is easily available thanks to the plantation turnover.

20 The bioequivalence examination was carried out for the substance bromeline, resulting in parity from 1% to 1% between bromeline and papaine.

The object of the present invention is a
"PHARMACEUTICAL COMPOSITION OF CARRIER SUBSTANCE FOR PRODUCTS
25 BASED ON VITAMIN-E, BROMELINE AND HYALURONIDASE".

The current technique comprises in the

pharmaceutical composition of the carrier substance the following formulation:

BROMELINEmore than 0.1%
HYALURONIDASE 50 to 900 utr/mg
5 VITAMIN-E 10 to 2000 mg

This technique was proofed through studies performed with 08 outpatients, in 02 distinct sessions of double blind analysis. The delimited area measured 15x10 cm with the application of gel, after 15 minutes the
10 measurements were started through liquid chromatography coupled to mass spectrometry.

The comparison of a control cream with the same active substance, for the purpose of calibration, yielded the following results regarding the cream according
15 to the present invention, with an analytical type of equal absorption area:

Control cream = area 131 vol. 0.54 = 8 hours;

Tested cream (invention) = area 131 vol. 0.54
= 1.5 hours.

20 The penetration relation equals to 100% of the required cream.

SOME SUBSTANCES TO BE CARRIED

- CUTANEOUS HEALERS
- PURE ANTIBIOTICS AND SULFA DERIVATIVES
- 25 - TOPICAL DERMATOLOGIC ANTI-FUNGUS AGENT
- TOPICAL RUBIFACIENT ANTIREUMATICS

- CORTICOSTEROIDS, ANTIMICOTICS, PURE AND ASSOCIATED ANTIBACTERICIDES
- TOPICAL ANTI-VARIX
- ANTI-HISTAMINIC ANTI-ITCH
- 5 - TOPICAL ANTIVIRALS
- TOPICAL LOCAL ANESTHETICS
- HORMONAL AND NON-HORMONAL ANTI-INFLAMMATORY
- HISTAMINE CLORIDRATE
- FILDENAFIL CITRATE
- 10 - FENTOLAMINA MESILATE
- ALPROSTADIL (Prostaglandine)

C L A I M S

1) "PHARMACEUTICAL COMPOSITION OF CARRIER
SUBSTANCE FOR PRODUCTS BASED ON VITAMIN-E, BROMELINE AND
HYALURONIDASE", characterized by the fact that it presents in
5 its formulation:

BROMELINEmore than 0.1%

HYALURONIDASE 50 to 900

utr/mg

VITAMIN-E 10 to 2000 mg

10 2) "PHARMACEUTICAL COMPOSITION OF CARRIER
SUBSTANCE FOR PRODUCTS BASED ON VITAMIN-E, BROMELINE AND
HYALURONIDASE", according to claim 1, characterized by the
fact that it presents in its formulation:

BROMELINE0,5 to 5%

15 HYALURONIDASE 50 to 900 utr/mg

VITAMIN-E 10 to 2000 mg

INTERNATIONAL SEARCH REPORT

International application No.
PCT/BR 01/00026

CLASSIFICATION OF SUBJECT MATTER

IPC⁷: A61K 38/47, 38/48, 31/355

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC⁷: A61K 38/47, 38/48, 31/355

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, CAS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 97/03676 A1 (CABO SOLER, J. ET AL.) 6 February 1997 (06.02.97) <i>claim 1.</i>	1,2
A	DE 19724458 A1 (MUCOS PHARMA GMBH & CO) 24 December 1998 (24.12.98) <i>claims 1,2,8.</i>	1,2
A	CH 630803 A5 (BEREMA S.A.) 15 July 1982 (15.07.82) <i>claims 1, 5, 6; page 5, left column, lines 25 - 35.</i>	1,2

☐ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

„A“ document defining the general state of the art which is not considered to be of particular relevance

„E“ earlier application or patent but published on or after the international filing date

„L“ document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

„O“ document referring to an oral disclosure, use, exhibition or other means

„P“ document published prior to the international filing date but later than the priority date claimed

„T“ later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

„X“ document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

„Y“ document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

„&“ document member of the same patent family

Date of the actual completion of the international search

7 June 2001 (07.06.2001)

Date of mailing of the international search report

3 July 2001 (03.07.2001)

Name and mailing address of the ISA/AT

Austrian Patent Office
Kohlmarkt 8-10; A-1014 Vienna
Facsimile No. 1/53424/535

Authorized officer

MOSSER

Telephone No. 1/53424/437

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. application No.

PCT/BR 01/00026

Patent document cited in search report			Publication date	Patent family member(s)			Publication date
CH	A	630803	15-07-1982	FR	A1	2433943	21-03-1980
				FR	A1	2515961	13-05-1983
				FR	A1	2515962	13-05-1983
				FR	A1	2520230	29-07-1983
				FR	B1	2515961	18-07-1986
				FR	B1	2515962	18-07-1986
				FR	B1	2520230	18-07-1986
				GB	A	1587638	08-04-1981
DE	A1	19724458	24-12-1998	none			
WO	A1	9703676	06-02-1997	ES	UA	1031261	16-12-1995